ISSN 0974 – 5211

Journal of Natural Products
Volume 3 (2010)
www.JournalofNaturalProducts.com

Research Paper

Antibacterial and Phytochemical Evaluation of Three Medicinal Plants

Akharaiyi Fred Coolborn* and Boboye Bolatito
Microbiology Department, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria
*Corresponding author
(Received 29 May 2009; Revised 04 June-11 July 2009; Accepted 08 August 2009)

ABSTRACT

The aqueos and ethanol extracts of the leaves, bark and roots of *Senna hirsuta, Landolphia dulcis* and *Daniella oliveri* were examined for possible sources of antimicrobial activities and phytochemical constituents. The preliminary evaluations of both the aqueous and ethanol extracts exhibited appreciable inhibitory activities on the tested pathogenic bacterial isolates at concentration of 20mg m/L. *S.hirsuta* showed more therapeutic activity where the aqueous extract of the leaves displayed inhibitory potency (30-37mm) in diameter on the tested bacterial isolates. The ethanol extract of the leaves showed inhibitory halo of between (30-38mm) in diameter. However, the leaves extracts of other plants as well demonstrated higher antibacterial potency than the bark and roots extracts. Saponins and alkaloids were recorded present in all the plants parts. While highest saponins constituent (5.10±0.11%) was recorded in the leaves extracts of *L.dulcis*, alkaloids was also highest in its leaves extract with a value of 2.98±0.12 %. The MIC of the extracts was similar in all the plants parts. The values ranged between 1.25-10mg/ ml.

Keyword: Antibacterial; Medicinal plants**.**

INTRODUCTION

Plants have a limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna, et al., 2007). The synthesized aromatic substances (Metabolites) are used by plants as defensive molecules against predation by microorganisms, insects and herbivores. However, some of which may involve in plant odour (terpenoides), pigmentation (tannins and quinines), and flavour (Capsacin) (Mallikharjuna, et al., 2007). However, these defensive molecules give plants their medicinal value which is appreciated by human beings because of their great importance in health care of individuals and communities.

Traditional medicine like orthodox medicine has its own methods and techniques of application which however aims at healing disease (Wurochekke, et al., 2008). The treatment and control of diseases by the use of the available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries of the world. Nearly, all cultures and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability. Due to ineffectiveness of most drugs as a result of microbial resistance to available agents most especially in developing countries, more patients are seen in medical centers than ever. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to suppress or possibly eradicate the ever increasing problems of emergence of newer diseases thought to be brought under control. In view of this, it is therefore very important to search for effective but of low cost and reliable traditional therapeutic agents, hence also the abuse of drugs for ailment is in high increase which motivated drug resistant organisms. This work is therefore aimed at studying three Nigerian medicinal plants used locally for treatment of some diseases for their phytochemical properties and antimicrobial activities in comparison to known antibiotics.

MATERIALS AND METHODS

***Plants Materials*:** Healthy leaves, stem bark and roots of *Senna hirusta, Daniella oliveri* and *Landolphia dulcis,* were collected from forest at Akure, Ondo State, Nigeria and identified by Dr Oyun, M.B. of the Department of forestry and wood technology, Federal University of Technology, Akure, Ondo State, Nigeria. The leaves were air dried and milled in a warring blender and sieved to obtain smooth powder.

***Bacterial Cultures**:* The bacteria strains used in this study are local isolates from urine, feaces and sore swab. The bacterial isolates include: *Pseudomonas aeruginosa*(sore), *Escherichia coli*(urine), *Staphylococcus aureus*(sore), *Salmonella typhi*(feaces), *Shigella dysenteniae*(feaces), *Klebsiella pneumoniae*(urine), *Bacillus cereus*(sore), and *Campylobacter jejumum*(feaces).

***Extraction*:** 1.5kg of each powdered plant part was extracted at room temperature (25 ± 2^0C) with ethanol and water for 48 h. The resulting mixtures were filtered and evaporated in a shaker water bath maintained at $55\text{-}65^0$C. The obtained dried crude extracts were contained in plastic containers and labeled appropriately as AE (aqueous extract) and EA (ethanol extract)

***Antibacterial Screening*:** The antibacterial activities of the crude plant extracts were evaluated by agar well diffusion (Nair and Chando, 2005). Nutrient agar (Lab M and Nutrient broth (Lab M) were used for the sub-culturing the bacterial isolates. Mueller-Hinton agar (Hi-media) was used for the sensitivity screening. The crude extracts were prepared in 5% v/v aqueous dimethyl sulphoxide (DMSO) at concentration of 20mg/ml. The inocula of the test bacterial isolates were prepared from 24h broth culture. The absorbance was read at 530nm and adjusted with sterile distilled water to match that of 0.5Mc Farland standard solution. From the prepared bacterial solutions, other dilutions were prepared to give a final concentration of 10^6 Colony Forming Unit (Cfu) per milliliter. 0.2ml each of the bacterial suspensions was obtained with sterile syringe and needle and spread plated on molten Mueller-Hinton agar. The plates were allowed to

stand for 1.5 h for the test bacterial isolates to be fully embedded and well established in the seeded medium. With a sterile cork borer wells of equal depth (Δ=5mm diameter) were dug with a previously sterilized No 4 cork borer. The wells were aseptically filled up with the extracts avoiding splash and overfilling. The plates were incubated at 37^0C for 24-48 h. The sensitivity of the test organisms to each of the extracts were indicated by clear halo around the wells. The halo diameters were taken as an index of the degree of sensitivity. Sterile 5% aqueous DMSO was used as negative control while streptomycin and methicilin (2mg/ml) was used as the positive control. All experiments were carried out in triplicates.

Minimum Inhibitory Concentration* (*MIC): The MIC was determined by the method of Sahm and Washington (1990). 1ml of the extract solution at concentration of 20mg/ml was added to 1mL of Muller-Hinton broth and subsequently transferred. 1mL from the first test tube to the next test tube and this continue up to the seventh test tube. Then 1ml of 24 h culture of the test bacteria organisms (1.0×10^6cell/ml) was inoculated into each test tube and mixed thoroughly. The test tubes were then incubated at 37^0C for 24 h. The tube with the lowest dilution with no detectable growth was considered as the MIC. For every experiment, a sterility check (5% v/v aqueous DMSO and medium) negative control (5% v/v aqueous DMSO, medium and inoculum) and positive control (5% aqueous DMSO, medium, inoculum and water soluble antibiotics) were included.

Statistical Analysis: The inhibitory zones of crude aqueous and ethanol extracts were expressed as the Mean±Standard Deviation and compared using student-Waller Ducan test at $P< 0.05$.

Phytochemical Screening

Preliminary Screening of secondary metabolites

***Saponin*:** 25g each of the powdered samples were boiled in 25ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Steroids: 2ml of acetic anhydride was added to 0.5g ethanol extract of each sample with the addition of 2ml H_2SO_4. A colour change from violet to blue or green indicates the presence of steroids.

***Flavonoids*:** A portion of the powdered plant samples were separately heated with 10ml of ethyl acetate in a water bath for 3min. The mixtures were filtered and 4ml of each filtrate were shaken with 1ml of dilute ammonia solution. A yellow colour observation indicates the presence of flavonoids (Harborne, 1973).

***Tannins*:** 0.5g of each powered samples were boiled in 20ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colour (Trease and Evans, 1989).

Total Phenol (Spectrophotometric methods): 2g each of the samples were defatted with 1ml of diethyl ether using a soxhlet apparatus for 2 h. The fat free samples were boiled with 50ml of ether for the extraction of the phenolic components for 15 minutes. 5ml of the extracts were pipetted into 5ml flask and then 10ml distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min. for colour development. This was measured at 505 nm.

Cardiac glycosides (Keller-Killam test): 5ml of each extracts was treated with 2ml of glacial acetic acid containing a drop of ferric chloride solution. This was under layed with 1ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

***Alkaloid*:** 5g of each sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 48 h. After filtration, the extracts were concentrated on a water bath to ¼ of the original volume. Concentrated ammonium hydroxide was added in drops to the extract until the precipitation was collected, washed with dilute ammonium hydroxide and then filtered. The residue obtained is the alkaloid, and was dried and weighed (Harborne, 1973).

Quantitative estimation of secondary metabolites

The presence of secondary metabolites from the leaves, roots and stem bark of the studied plants were quantitatively determined by adopting standard protocols. Saponins by Obadoni and Ochuko, 2001; flavonoids by the criteria of Boham and Kocipai-Abyazan, 1974; tannis by the criteria of Van-Burden and Robinson, 1981; alkaloids was determined by the methods of Ikan, 1981 and phennols by the method of Bray and Thorpe, 1964.

RESULTS

The three plants studied revealed the presence of bioactive properties in their parts (leaves, stem bark and roots) in various degrees. Local users of these plants employ primarily water as solvent of extraction for the preparation of infusions and decoctions in different parts of Nigeria. *Landolphia dulcis* among the three plants exhibited more antimicrobial activities (Tables-1A, 1B and 1C).

Table-1A: Mean inhibitory halo (mm) as expressed by the crude aqueous and ethanol extracts of the plants at 20mg/ml.

Bacterial organisms	*Senna hirsuta*						References	
	Leaves		Bark		Root		Strept.	Methy.
	AE	EE	AE	EE	AE	EE		
S. dysenteriae	30.17±1.6	36.63±1.6	22.01±0.6	32.11±1.6	18.0±0.6	20.00±1.6	40.23±1.0	20.00±1.0
P. aeruginosa	33.18±0.6	35.54±0.6	20.11±0.6	18.32±1.6	19.13±0.6	20.71±1.6	39.18±1.0	15.0±1.0
E. coli	33.06±1.6	36.20±1.6	20.09±0.5	22.16±0.6	12.36±1.6	17.66±1.6	0.0±1.0	0.00±1.0
S. typhi	32.10±1.6	38.33±0.5	21.84±0.5	18.15±0.5	14.12±1.6	18.47±1.6	0.0 ±0.0	12.11±0.0
B. cereus	31.18±1.6	35.73±0.6	17.12±0.6	25.36±1.6	11.72±1.6	19.46±1.6	40.00±0.0	18.6±1.0
S.aureus	37.15±1.6	30.66±1.6	30.24±0.5	35.53±1.6	10.14±0.5	19.35±1.6	45.16±1.0	0±0.0
C. jujenum	34.16±1.6	38.60±1.6	22.16±0.5	26.06±1.6	13.10±0.5	16.12±1.6	0±0.0	0.0±0.0
K. pneumoniae	32.25±0.5	37.40±1.6	30.28±0.5	32.55±1.6	22.14±0.5	28.43±0.5	0.0±0.0	0.0±0.0

- The results are the mean values of triplicate tests measured in two directions after 24-48h incubation at 37^0C.
- Streptomycin and Methycilin at concentration of 2mg/ml were used as the positive reference drugs.
- AE: Aqueous extracts, EE: Ethanol extracts.

Table-1B: Mean inhibitory halo (mm) as expressed by the crude aqueous and ethanol extracts of the plants at 20mg/ml.

Bacterial organisms	*Daniella oliveri*					
	Leaves		Bark		Root	
	AE	EE	AE	EE	AE	EE
S. dysenteriae	16.19±1.6	24.76±1.6	12.24±0.6	29.14±0.6	8.64±1.6	16.18±0.6
P .aeruginosa	30.13±1.6	34.72±1.6	18.45±0.6	24.12±1.6	25.22±0.6	30.64±1.6
E. coli	30.19±1.5	36.35±1.5	28.18±0.5	31.62±1.6	10.51±1.6	13.18±1.6
S. typhi	33.18±0.5	37.60±1.6	22.00±0.5	28.11±0.5	12.40±1.6	9.08±0.5
B. cereus	20.14±1.6	26.00±0.5	12.34±0.5	16.30±1.6	6.12±0.5	9.26±0.5
S.aureus	24.18±1.6	27.11±0.5	22.30±0.5	26.13±1.6	10.00±0.6	15.34±1.6
C. jujenum	18.12±1.6	38.74±1.6	20.14±0.5	25.16±1.6	16.13±0.5	14.70±1.6
K. pneumoniae	25.18±0.6	38.74±1.6	26. 35±1.5	30.46±1.6	18.30±1.6	21.18±1.6

- Streptomycin and Methycilin at concentration of 2mg/ml were used as the positive reference drugs as shown in table-1A.
- Other details are same as shown in table-1A.

Table-1C: Mean inhibitory halo(mm) as expresses by the crude aqueous and ethanols of the plants at 20mg/ml.

Bacterial organisms	*Landolphia dulcis*					
	Leaves		Bark		Root	
	AE	EE	AE	EE	AE	EE
S. dysenteriae	24.32±0.5	35.68±1.6	18.04±0.6	22.63±1.6	10.15±0.6	14.26±0.6
P. aeruginosa	35.20±1.6	38.18±0.6	32.00±0.6	34.52±1.5	30.08±0.6	36.14±0.6
E. coli	32.19±0.5	36.28±0.6	28.17±1.6	31.56±1.5	12.15±0.6	34.66±1.6
S. typhi	31.39±1.5	37.62±1.6	23.13±0.5	30.10±0.5	20.54±1.6	18.60±1.6
B. cereus	28.35±0.5	36.55±0.5	31.44±1.6	37.66±1.6	17.14±1.6	21.24±0.5
S. aureus	30.33±1.6	33.71±1.6	16.34±1.6	14.18±0.6	18.19±0.6	24.61±0.6
C. jujenum	32.14±0.5	36.47±1.6	30.11±1.6	36.65±1.6	14.16±1.6	29.34±1.6
K. pneumoniae	35.52±0.5	38.71±1.6	31.26±0.6	34.16±0.6	23.30±0.6	28.72±0.6

- Streptomycin and Methycilin at concentration of 2mg/ml were used as the positive reference drugs as shown in table-1A.
- Other details are same as shown in table-1A.

Alkaloids and saponins were present in all the screened plant parts. This was followed by flavonoids which was not present on the root of *Daniella oliveri* and glycocides which was also absent in the leaf (Table-2) based on the type of test carried out on them.

Table-2: Qualitative estimation of secondary metabolites from the plants parts used.

Secondary metabolites	*Senna hirsuta*			*Daniella oliveri*			*Landolphia dulcis*		
	LF	SB	RT	LF	SB	RT	LF	SB	RT
Saponins	±	±	±	±	±	±	±	±	±
Steroids	±	±	±	±	±	±	±	-	±
Flavonoids	±	±	±	±	±	-	±	±	±
Tannins	±	±	-	-	±	-	±	±	±
Glycosides	±	±	±	-	±	±	±	±	±
Alkaloid	±	±	±	±	±	±	±	±	±
Phenols	±	±	±	±	±	-	±	±	±

- LF = Leave, SB = Stem bark, RT = Root.

Though all these were found to be more abundant in distribution, the quantitative analysis carried out showed that saponin was highest in *Landolphia dulcis.* Though flavonoids and phenols percentage as recorded, were extremely low, they were widely distributed in all the plants used except in the roots of Daniella oliveri where it was absent (Table-3). The reference antibiotics (streptomycin and methicilin) as the positive control in concentration of 2mg/ml as used in this study though higher in inhibitory affinity (39-45mm) [streptomycin] and lower in inhibitory affinity (12-20mm) [methicilin] were not active (0mm) on *E.coli, S.typhi, C. jejumum* and *K. pneumoniae*. Despite this position, these organisms were quite susceptible to the plant extracts though at higher concentrations than the reference drugs.

The MIC of the extracts against the tested bacterial organisms is shown in tables 4A and 4B.

Table-3: Quantitative estimation of secondary metabolites from the plant parts used.

Percentage of secondary metabolites (%)									
Secondary metabolites	***Senna hirsuta***			***Daniella oliveri***			***Landolphia dulcis***		
	LF	**SB**	**RT**	**LF**	**SB**	**RT**	**LF**	**SB**	**RT**
Saponins	4.3±0.1	2.3±0.1	1.84±0.1	3.92±0.1	0.46±0.0	0.26±0.2	5.10±0.1	2.56±0.1	2.24±0.1
Flavonoid	0.34±0.1	0.88±0.2	0.25±0.1	0.56±0.2	0.76±0.3	-	0.89±0.1	0.99±0.1	0.74±0.2
Tannins	22.13±0.1	20.20±0.1	-	-	16.13±0.2	-	20.85±0.3	20.35±0.1	20.98±0.3
Alkaloid	1.70±1.2	1.82±0.2	1.71±0.3	1.46±0.0	1.88±0.0	183±0.1	2.98±1.2	2.24±0.1	2.04±0.2
Phenols	0.10±0.0	0.16±0.3	0.20±0.1	0.04±0.1	0.08±0.2	-	0.16±0.1	0.64±0.2	0.84±0.2

- Results are the mean of triplicate tests.

Table- 4A: Minimal inhibition concentrations (MIC) of the plants extracts.

Bacterial organisms	***S. hirsuta***						***D. oliveri***					
	Leave		**Bark**		**Root**		**Leave**		**Bark**		**Root**	
	AE	**EE**	**AE**	**EE**	**AE**	**EE**	**AE**	**EE**	**AE**	**EE**	**AE**	**EE**
S. dysenteriae	1.2	1.2	10	5	10	10	10	10	10	10	10	10
P. aeruginosa	1.2	1.2	5	5	10	10	2.5	1.2	5	5	10	5
E. coli	1.2	1.2	5	5	10	10	2.5	2.5	5	5	2.5	2.5
S. typhi	1.2	1.2	5	5	10	10	5	5	5	2.5	10	10
B. cereus	1.2	1.2	5	5	10	10	5	5	5	5	10	10
S .aureus	1.2	1.2	5	5	10	10	5	5	10	10	10	10
C. jejunum	1.2	1.2	10	2.5	10	10	5	5	10	10	10	10
K. pneumoniae	1.2	1.2	5	5	5	2.5	5	5	5	5	10	10

- Streptomycin and Methycilin at concentration of 2mg/ml were used as the positive reference drugs as shown in table-4B. Other details are same as shown in table-1A.

Table- 4B: Minimal inhibition concentrations (MIC) of the plants extracts.

Bacterial organisms	***L. dulcis***						**References**	
	Leaves		**Bark**		**Root**		**Strept.**	**Methy.**
	AE	**EE**	**AE**	**EE**	**AE**	**EE**		
S. dysenteriae	10.00	10.00	2.5	5.0	10.0	10.0	0.125	1.0
P. aeruginosa	1.25	1.25	2.5	1.25	1.25	1.25	0.125	1.0
E. coli	1.25	1.25	2.5	1.25	10.0	1.25	-	-
S. typhi	1.25	1.25	1.25	1.25	10.0	5.0	-	-
B. cereus	1.25	1.25	5.0	1.25	10.0	5.0	0.125	1.0
S.aureus	1.25	1.25	2.5	2.5	10.0	5.0	0.125	1.0
C. jujenum	1.25	1.25	2.5	2.5	5.0	5.0	-	-
K. pneumoniae	1.25	1.25	2.5	2.5	5.0	5.0	-	-

- Streptomycin and Methycilin at concentration of 2mg/ml were used as the positive reference drugs. Other details are same as shown in table-1A.

DISCUSSION

Based on the present study, we can consider the plants understudied of the *Leguminasiae* family (*Senna hirsuta* and *Daniella oliveri*); and *Apocynaceae* family (*Landolphia dulcis*) leaves, stem bark and roots to be good sources of antimicrobial property. This results obtained conforms to the findings of Wurochekko et al. (2008). The bioactive compounds on the medicinal plants employed contain various secondary metabolites such as phenols, tannins, alkaloids, flavonoids, steroids and glycosides in appreciable quantities. The effective inhibitory potency observed with the plants parts; proof it that the inhibitory compounds were extractable by the employed solvents against the tested pathogenic bacterial isolates. This observation as reported correlates with De and James (2002) who emphasized that these compounds are known to show medicinal activity as well as exhibiting physiological activity.

However, the extracts of the plants parts were used at a concentration of 20mg/ ml in the respective solvents. This concentration was visibly active on the tested bacterial isolates due to the combinative therapeutic actions of the various secondary metabolites contained in the plants. Some of the tested bacterial isolates such as *S.dysentariae, P aeruginosa, E. coli* and *K. pneumoniae* reported to be associated with nosocomial and community acquired infections (Indrayan, et al., 2002; De and James, 2002) were found susceptible to the plants crude extracts used in this study. This proof emphasized that some nosocomial and community aquired infections could be prevented or alleviated with the use of most especially, the ethanol extracts of the studied plants. These plants, most especially their leaves and stem bark in dry powder form could be used for direct consumption as various kinds of beverages, decoctions and infusions. Healthy fresh leaves of these plants could as well be prepared as soup.

The percentage MIC values of the plants were based where both the ethanol and aqueous extracts have the same MIC values on the tested bacterial isolates. This perhaps helps to interpret that differences in inhibitory diameters (mm) could result in the same therapeutic potency when varied in concentrations, depending on the organism's susceptibility to the antibacterial components present in the extracts. The presence and the phytochemical components of the studied plants, the inhibitory zones and the MIC concentrations at which values were effective on the tested organisms, highlights that there were variations in the antibacterial potency of the plants extracts. The variations in the sensitivity could also be attributed to the differences in growth rate of the tested organisms, nutritional requirements, temperature and inoculum size (Gaill and Jon, 1995).

It has been reported that antibiotics are not the only antibacterial agents and this study observed the effective potency of the studied plants extracts on the selected pathogenic bacterial isolate than some highly rated antibiotics (reference drug) in disease cure and prevention. One problem in the use of medicinal plants is the quantity desired to effect cure hence most times, medication is basically on unspecified quality of decoctions and infusions. Irrespective of the plants parts in this study and methods of extraction (ethanol and water), a dosage of between 1.2-10mg exhibited appreciable inhibitory values on the tested bacterial species.

CONCLUSION

The plants parts antibacterial effectiveness on the tested bacterial isolates resulted within 24h of incubation in both the crude extract screening and MIC values. The aqueous extracts of the plants displayed extensively a competitive inhibitory potency with the more effective ethanol extracts of the plants parts on the tested isolates which majority are Gram negative bacteria known for their ability to form resistance to drug. The plants parts though effective on all the bacterial isolates, there were variations in inhibitory potency resulting from variations in the secondary metabolites concentrations in the plants parts.

REFERENCES

Boham, B.A., Kocipai-Abyazam, R., (1974): Flavonoids and condensed tannins from leaves of *Hawaiina vacinium* vaticulatum and *V. Calycinium. Pacific Sci.,* 48:458-463.

Bray, H.G., Thorpe, W.V., (1964): Meth. Biochem. *Anal.,* 1:27.

De, N., James, N.E., (2002): Antibacterial spectrum of ectracts of *Ocimum gratissimum* L (Basil) ND *Xylopia aetiopica* A. Rich (Dunal). *Nig. J. Appl. Sci*., 11: 165-175.

Gaill, W., Jon, A.W., (1995): Antimicrobial susceptibility test; dilution and disc diffusion methods. Manual of Clinical Microbiology, 6th Edn., pp.1327-1332.

Harborne, J.B., (1973): Phytochemical methods, London. Chapman and Hall, Ltd., Pp. 49-188.

Ikan, R., (1981): Natural products: A laboratory guide, Academic Press, London.

Indrayan, A.K., Shama, A., Gideria, B.S., Gupta, C.P., (2002): Antibacterial activity of dye from ensalpina sappan (Patang/Brazilworld). *In. J. Microbiol*., 42: 359-360.

Mallikharjuna, P.B., Rajanna, L.N., Seetharam, Y.N., Sharanabasappa, G.K., (2007): Phytochemical studies of *Strychnos potatorum* L.f. A medicinal plant. *E-jour. Chem*., 4 (4): 510-518

Nair, R., Chando, S., (2005): Anticandida activity of Punica granatum exhibited in different solvents. *Pharm. Biol*., 42: 21-25.

Obadoni, B.O., Ochuko, P.O., (2001): Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Glob.J. Pure Appl. Sci*., 86: 2003-2008

Sahm, D.F., Washington, J.A., (1990): Antibacterial susceptibility test dilutions methods: In: Manuals of clinical Microbiology.

Trease, G.E., Evans, W.C., (1989): Pharma cognsy. 11th Edn., Brailliar Tiridel Can. Macmillan Publishers.

Van-Burden, T.P., Robinson, W.C., (1981): Formation of Complexes between protein and tannin acid. *J. Agric. Food Chem.,* 1:77.

Wurochekke, A.U., Anthony E.A., Obadiah W., (2008): Biochemical effects on the liver and kidney of rats administered aqueous stem bark extract of *Xemenia Americana*. *African Biotech*., 7(16): 2777-2780.

